Title

Amyotrophic lateral sclerosis of long clinical course clinically presenting with progressive muscular atrophy

Running Title

ALS clinically presenting with PMA

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Abstract

Amyotrophic lateral sclerosis (ALS) primarily affects upper and lower motor neurons. Phosphorylated TAR DNA-binding protein of 43 kDa (TDP-43) inclusion bodies are reportedly a pathological hallmark of sporadic ALS. Here, we present an atypical case of sporadic ALS that progressed very slowly, persisted for 19 years, and clinically appeared to only affect the lower motor neurons; however, upper motor neuron degeneration was detected on autopsy. Furthermore, no inclusion bodies positive for phosphorylated TDP-43, ubiquitin, fused in sarcoma, or SOD1 were detected in the CNS. We performed exome-sequencing data analysis but found no genetic disorders. This was therefore an unusual case of lower motor neuron-predominant ALS without TDP-43 pathology or known gene-disease associations. We also reviewed autopsied ALS cases that progressed slowly and had no phosphorylated TDP-43 or ubiquitin positive inclusions and present the clinicopathological features of such cases. Based on these results, there may be a sporadic

ALS subgroup that progresses slowly and shows no accumulation of phosphorylated TDP-43.

Key words

Amyotrophic lateral sclerosis, Progressive muscular atrophy, Motor neuron disease, Autopsy, TDP-43

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects upper and lower motor neurons.[1] Recently, phosphorylated TAR DNA-binding protein of 43 kDa (TDP-43) was identified in intracytoplasmic inclusion bodies of ALS, and the accumulation of TDP-43 is considered a major feature of sporadic ALS.

Progressive muscular atrophy (PMA), also referred to as spinal progressive muscular atrophy,[2] progressive spinal muscular atrophy,[3] or Aran-Duchenne disease[4] is characterized by isolated signs of lower motor neuron (LMN) dysfunction, loss of anterior horn cells, and preservation of upper motor neuron (UMN) and corticospinal tracts.[1] Phosphorylated TDP-43-immunopositive inclusions were also observed in PMA.[5] Therefore, sporadic ALS and PMA are now considered to fall within the same disease spectrum as examples of TDP-43 proteinopathy.

Here, we present an autopsy case of sporadic ALS clinically mimicking PMA, but our case had no evidence of phosphorylated TDP-43-positive inclusion bodies or known gene disorders. This is an ALS case outside the category of TDP-43 proteinopathy, and thus provides new insight into the classification of ALS.

Clinical Summary

A 55-year-old, right-handed Japanese woman developed muscle weakness bilaterally in the proximal part of her lower extremities. Her body weight had decreased by 3 kg in 5 months. Subsequently, muscle weakness spread to the upper extremities and distal part of the lower extremities. By the age of 59 years, the patient was unable to climb the stairs or use chopsticks or a pen. The patient's medical history included mild hypertension and a partial colectomy with spleen cholecystectomy, for colon cancer, performed at the age of 54 years. She had no family history of neurological disease. Additionally, there was no history of consanguineous marriage.

A neurological examination at the age of 60 years revealed diffuse muscle atrophy in the upper and lower extremities and trunk, as well as decreased deep tendon reflexes in the extremities. There was no cognitive dysfunction, cranial nerve symptoms, pathological reflex, extrapyramidal symptoms, ataxia, major sensory deficit, or autonomic dysfunction. Laboratory data were normal, except for serum creatine kinase levels and serum lactate dehydrogenase levels, which were recorded at 710 IU/ml and 486 U/ml, respectively. Upper and lower limb motor nerve conduction analysis revealed mildly reduced compound muscle action potential amplitudes and normal conduction velocities, with no evidence of conduction block or dispersion. The sensory nerves were normal. Needle electromyography showed both active and chronic denervation in the cervical and lumbosacral regions and only chronic denervation in the bulbar region. Brain MRI results showed mild periventricular white matter ischemic changes. Cervical MRI results revealed mild spinal canal stenosis at the level of C5/6.

Because atypical ALS with gene abnormalities was considered in the differential diagnosis, we performed a genetic analysis of this case. This investigation was approved by the institutional ethics committee of Hiroshima University. Written informed consent was obtained from the patient. Genetic studies, including whole-exome sequencing (WES),

demonstrated no pathological variation in the genes currently known to be associated with ALS or spinal muscular atrophy (SMA). No hexanucleotide repeat expansion was detected in C9orf72.

Dyspnea gradually progressed and non-invasive positive pressure ventilation was initiated at the age of 65 years and used throughout the day at the age of 73 years. On the other hand, the patient's sonographic tongue thickness was 46.1 mm [healthy control 44.8 ± 3.0 mm; ALS 41.9 ± 4.0 mm (mean ± SD)[6]], and she showed no clinical signs of tongue atrophy. In addition, the patient maintained oral intake and cognitive function almost throughout the clinical course of the disease. The patient died from respiratory failure at the age of 74 years, and an autopsy was performed. The clinical diagnosis was PMA. The total duration of the disease was 19 years.

Pathological Findings

The autopsy was performed 9.6 hours after death, and the tissues were fixed in 20% buffered formalin. The left cerebral hemisphere was dissected in the coronal plane. The right hemisphere above the anterior commissure-posterior commissure (AC-PC) line was dissected in the sagittal plane for sufficient observation of the precentral gyrus. The right hemisphere below the AC-PC line was dissected in the axial plane for observation of the posterior limb of the internal capsule. The brainstem and right cerebellum were dissected in the axial plane, and the left cerebellum was dissected in the sagittal plane.

Six-micrometer-thick paraffin-embedded sections were stained with HE, KB, and Gallyas-Braak stains. Furthermore, we performed immunostaining with the primary antibodies listed in Table 1.

The brain weight was 1100 g after fixation. Macroscopic examination revealed selective atrophy of the ventral spinal roots, relative sparing of the dorsal spinal roots,

and atrophy of the anterior horn. In the cerebrum, mild atrophy of the precentral gyrus was observed (Fig. 1A). In the brainstem, atrophy of the medullary pyramid was observed. The cerebellum had no remarkable findings.

Microscopically, the anterior and lateral columns showed a bilateral decrease in myelin sheath staining compared with the posterior column (Fig. 1B). There was almost total neuronal loss in the spinal anterior horn at all levels of the spinal cord (Fig. 1C–E), but relative sparing of the Onufrowicz nuclei and Clarke's column. In the brainstem, slight left-dominant atrophy of the medullary pyramid was also observed (Fig. 1F). Although myelin pallor and axonal loss of the pyramid were not obvious, CD68-immunopositive microglia/macrophages were observed to a higher degree than in other areas. Although the cranial motor nuclei were relatively spared, mild but definite gliosis was observed at the hypoglossal, facial, and trigeminal motor nuclei (Fig. 1G–I). Betz giant cells were mildly reduced and some neuronophagia was observed in the primary motor area of the sagittal section (Fig. 1J, K). The inferior olive nuclei, medullary reticular formation, substantia nigra, putamen, and globus pallidus were relatively preserved. No phosphorylated TDP-43-positive intracytoplasmic inclusion bodies, ubiquitinated intracytoplasmic inclusion bodies, or Bunina bodies were detected in the CNS. Likewise, no notable fused in sarcoma (FUS) or SOD1 immunoreactivity was detected in the CNS.

Small amounts of senile plaques, NFTs, and argyrophilic grains were detected in restricted regions. These pathological features were consistent with Braak amyloid stage A,[7] Thal phase for amyloid β deposition I,[8] Consortium to Establish a Registry for Alzheimer’s Disease score A,[9] Braak NFT stage II,[7] Braak AT8 stage II,[10] and argyrophilic grains Saito stage II.[11] No α-synuclein pathology was detected.

Discussion

This report described a very slowly progressing ALS case that clinically appeared to only affect the LMNs except for the cranial nerve area. Postmortem pathological investigation was able to reveal mild degeneration in UMN and brainstem motor neuron nuclei as well as degeneration in anterior horn cells. These pathological findings were consistent with ALS. Notably, this ALS case showed no intracytoplasmic accumulation of phosphorylated TDP-43.

In this case, signs of UMN degeneration were not clinically obvious throughout the clinical course of the disease. We had difficulty in diagnosing whether this was a case of ALS or another condition primarily affecting the LMNs. As seen in the present case, severe LMN degeneration can clinically mask signs of UMN degeneration, which is why most (84.6%) of patients clinically diagnosed as PMA show both UMN and LMN degeneration through pathological examination.[5] In other reports, pathological examination also revealed UMN degeneration in 2/2 cases[12] and 1/2 cases[13] of clinically-diagnosed PMA.

As a pathological feature of this case, no inclusions immunopositive for phosphorylated TDP-43 were observed. Immunohistologically, ubiquitinated intracytoplasmic inclusion bodies are known as a major pathological hallmark of ALS. In 2006, phosphorylated TDP-43 was identified as a component of these ubiquitinated inclusion bodies in sporadic ALS.[14,15] With the exception of some familial ALS, including cases with *SOD1* and *FUS* mutations,[16-18] most ALS shows intracellular aggregation of phosphorylated TDP-43 as a prominent pathological feature. Subsequent studies showed that phosphorylated TDP-43 inclusion bodies were also observed in PMA cases.[5] However, our case was atypical in that no inclusion bodies immunopositive for either phosphorylated TDP-43, SOD1, FUS, or ubiquitin were observed although other pathological findings were consistent with ALS. Slowly progressive ALS cases without phosphorylated TDP-43 or ubiquitin positive intracytoplasmic inclusions have been rarely reported.[2, 19-21] We reviewed

five cases and compared them with the current case in terms of their clinicopathological features (Table 2). In general, the median survival time of ALS patients was reported as 20–48 months,[22] and long-surviving ALS cases are not common. However, the mean disease duration from disease onset to death or mechanical ventilation of the cases in Table 2 was 16 years, which is considerably longer than the typical disease duration of sporadic ALS. These cases showed relatively mild bulbar signs and half of the cases did not show pyramidal signs. The similarity of these cases suggests the existence of a slowly progressing sporadic ALS subgroup unrelated to the accumulation of phosphorylated TDP-43. As a differential diagnosis for our patient, we considered atypical ALS with genetic mutation or other motor neuron degenerative diseases with genetic mutation. To support this idea, in a previous study, a survival motor neuron-deficient mouse model showed no TDP-43 pathology.[23] However, our case showed no pathological variation in the genes currently known to be associated with ALS or SMA.

As another pathological feature of this case, UMN degeneration was very mild. If proper pathological examination had not been conducted, the UMN degeneration, especially in the primary motor area, may not have been detected and a diagnosis of ALS may not have been possible. In this case, we examined the sagittal section of the precentral gyrus in the left cerebral hemisphere and the coronal section of the precentral gyrus in the right cerebral hemisphere. Several instances of neuronophagia were observed in the sagittal section, but typical neuronophagia was not detected in the coronal section with HE staining; possibly because the sagittal sections vertically correspond to the precentral gyrus and enable the observation of a wider area than do coronal sections. Accordingly, sequent sagittal sections may enable the detection of UMN degeneration with increased sensitivity.

In conclusion, we report an atypical case of LMN-predominant ALS without TDP-43 pathology or known gene-disease associations. There may be a sporadic ALS subgroup that progresses slowly and shows no accumulation of phosphorylated TDP-43.

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Disclosure

The authors declare that they have no conflict of interest.

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Figure Legends:

Figure 1: Mild atrophy of the precentral gyrus in the right hemisphere (A). The bilateral anterior and lateral column showing decreased KB staining compared with the posterior columns in the cervical spinal cord (B). Lumbar spinal cord showing almost total neuronal loss of anterior horn cells (C) and severe gliosis as shown by HE staining (D) and anti-GFAP antibody immunostaining (E). Atrophy of the medullary pyramid, showing possible left dominance with KB staining (F). Hypoglossal motor nuclei showing relative sparing of neurons (G) and mild gliosis, as shown by HE staining (H) and anti-GFAP antibody immunostaining (I). Neuronophagia in the primary motor area, as shown by HE staining (J). The CD68-immunopositive microglia/macrophage in the primary motor area (K). Scale bars: (A) 1 cm, (B, F) 2 mm, (D, E, H–K) 50 μm, (C, G) 200 μm.

Figure 1 A-E

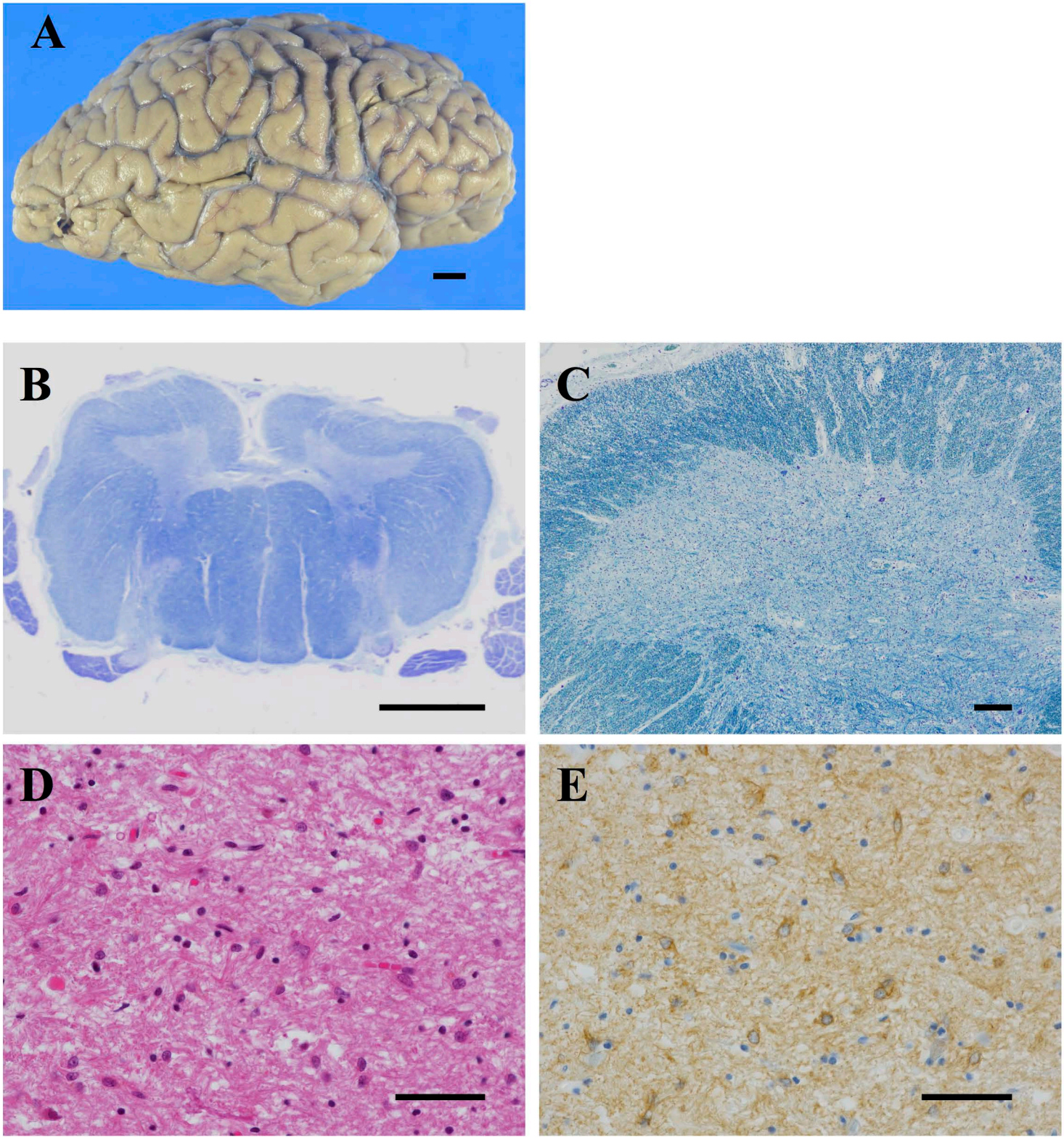

Figure 1 F-K

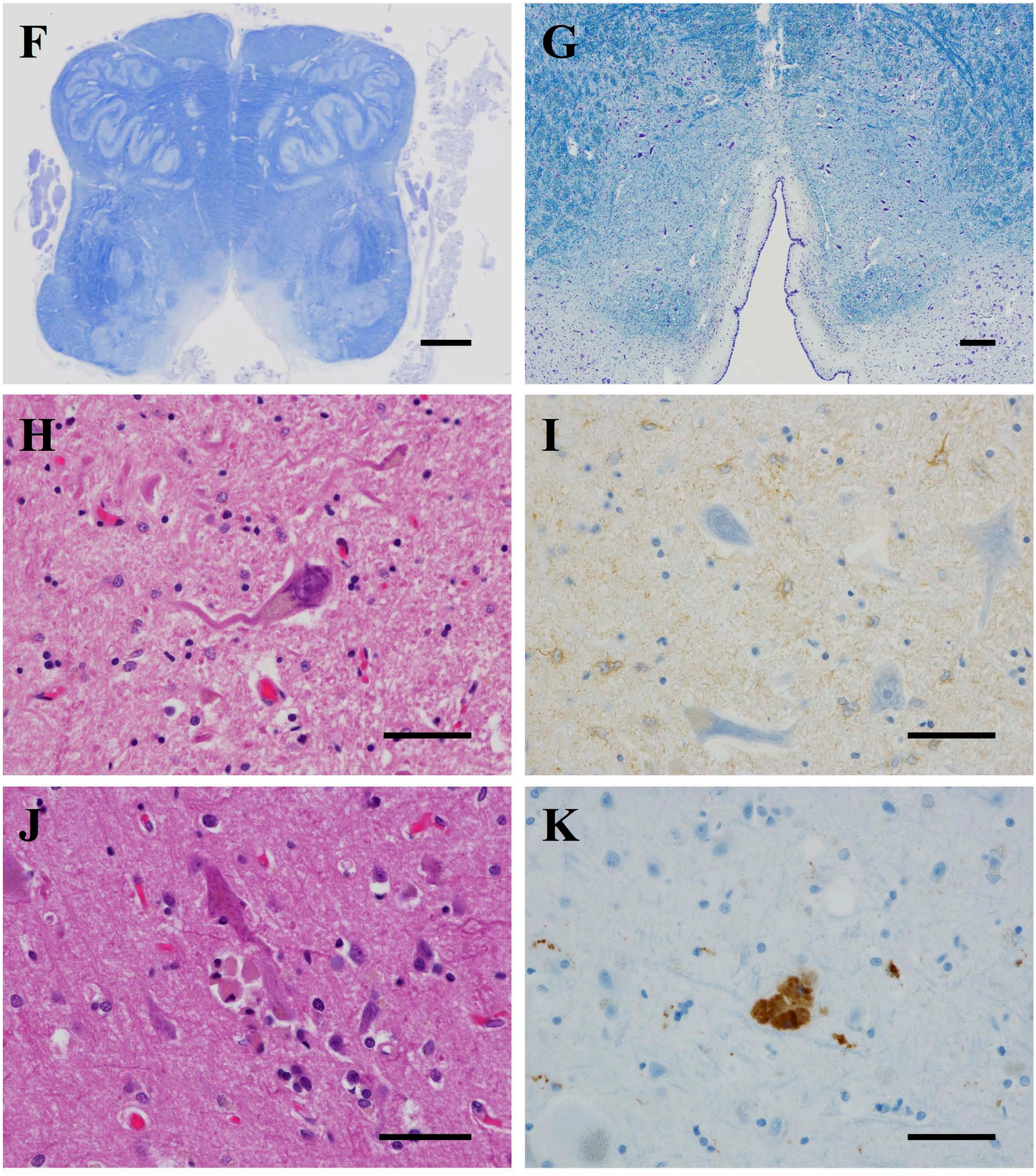

Table 1 Antibodies used for immunohistochemistry

Primary antibodies	Host	Dilution	Source
phosphorylated TDP-43 (pSer409/410)	mouse-monoclonal	1:10000	A gift from M. Hasegawa, Japan
ubiquitin	rabbit-polyclonal	1:1000	Dako, Glostrup, Denmark
fused in sarcoma (FUS)	rabbit-polyclonal	1:200	Sigma-Aldrich, St Louis, MO, USA
Cu/Zn superoxide dismutase-1 (SOD1) (1G2)	mouse-monoclonal	1:2000	MBL, Aichi, Japan
GFAP	rabbit-polyclonal	1:10	Dako, Glostrup, Denmark
human macrophage CD68 (PG-M1)	mouse-monoclonal	1:100	Dako, Glostrup, Denmark
phosphorylated neurofilament (SMI31)	mouse-monoclonal	1:20000	Sternberger Monoclonals Inc., Baltimore, MA, USA
phosphorylated tau (AT8)	mouse-monoclonal	1:1000	Innogenetics, Gent, Belgium
human amyloid β 11-28 (12B2)	mouse-monoclonal	1:50	IBL, Maebashi, Japan
phosphorylated α-synuclein (pSyn#64)	mouse-monoclonal	1:10000	A gift from T. Iwatsubo, Japan

Table 2 Comparison among slowly progressive ALS cases without TDP-43 or ubiquitin positive intracytoplasmic inclusions

Case No	1	2	3	4	5	6
Reference	Tsuchiya et al.[19]	Honma et al.[20]	Tsuchiya et al.[2]	Mochizuki et al.[21]	Mochizuki et al.[21]	Current case
Year	1999	1999	2004	2016	2016	
Clinical findings						
Sex	F	M	F	M	M	F
Age at onset (years)	42	49	52	48	55	55
Age at death (years)	61	65	71	76	84	74
Disease duration (years)	19	16	19	28	29	19
Duration from onset to NPPV (years)	none	none	none	none*	none*	10: sometimes, 18: always
Duration from the onset to tracheostomy (years)	15	none	none	8	14	none
Duration from the onset to mechanical ventilation (years)	15	none	none	13: sometimes, 25: always	14	none
Initial symptom	weakness of the left lower extremity	weakness of the right hand	weakness of the right upper extremity	weakness of the right hand	weakness of the left hand	weakness of the bilateral lower extremity
Pyramidal sign	-	+	-	+	+	-
Bulbar sign	mild	mild	mild	evident*	evident*	none
Dementia	not described	none	not described	none	none	none
Cause of Death	sudden respiratory arrest	respiratory failure	sudden respiratory arrest	malignant lymphoma	sudden death	respiratory failure
Pathological findings						
Brain weight (g)	1230	1410	1130	1190	1030	1100
Loss of Betz cells	observed	mild	observed	evident	evident	mild
Degeneration of pyramidal tract	evident	mild	evident	mild	mild	mild
Loss of motor neuron nuclei in the brainstem	slight	evident	evident	moderate	moderate	mild
Loss of anterior horn cells	evident	evident	evident	moderate	moderate	evident
Ubiquitinated inclusion	negative	negative	negative	negative*	negative*	negative
TDP-43-positive inclusion	not described	not described	not described	negative	negative	negative
Bunina body	negative	negative	negative	negative	negative	negative

F: female, M: male, NPPV: noninvasive positive-pressure ventilation

* information from personal communication with Y. Mochizuki